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RESEARCH ARTICLE

A REPORT ON THE STABILITY OF HERBAL SYNTHESIZED AG NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

An ecological method of green mediated synthesis of nanoparticles is significant step in the field of nanotechnology. In this method we have examined green synthesis of Ag nanoparticles using a mixture of Salix viminalis leaves extract as a reducing agent and surfactant (CTAB) as stabilizer. These green synthesized nanoparticles characterized with the help of UV–vis spectrophotometer, DLS, FTIR, and TEM. Nanoparticle size distribution was determined within 1 to 100nm by TEM and DLS and the optimum wavelength was recorded in 400 to 450nm by UV-vis spectroscopic studies. The chemical groups readings using FT-IR analysis. FT-IR spectrum shows that the nanoparticles are destined to protein through the carboxylate ion group. In this study we observed that S. viminalis leaf extract reduce Ag ion in to Ag nanoparticles within 10mins of reaction time which indicated that it is a rapid synthesis. These synthesized Ag nanoparticles showed good antibacterial properties against both E. coli (gram -ve) and S. aureus (gram +ve) with the maximum 11mm inhibition zone. Additionally we have examined that synthesized Ag nanoparticles are quite stable and remain intact for approximately three months. This is the most useful & interesting step in this work.

INTRODUCTION

The most successfully studied nanoparticles now in days made from novel metals, in particular Ag, Pt, Zn and Au. The field of nanotechnology is one of the liveliest researches nowadays in modern material science and technology. Among the methods involved in synthesis of metal nanoparticles, biological methods of synthesis are currently gaining importance and dependable due to its cost effective, eco-friendly and usage of non-toxic materials in the processing (Jayaraman, 2013). The formation of gold and Ag nanoparticles by plants for the first time reported (Gardea-Torresdey, 2003 and Vankar, 2012). The formation of bacterium-mediated nanoparticles is eco-friendly and economically viable process (Deshmukh, 2008). Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is their exhibit larger surface area to volume ratio (Leela, 2008 and Lalitha, 2013). Nanoparticles shows completely new and improved properties based on specific characteristics such as size, distribution and morphology. Green synthesis of nanoparticles is advantageous over physical and chemical methods as it is a cost effective and environment friendly method, where it is not necessary to use high pressure, energy, temperature and toxic chemicals (Sinha, 2009 and Goodsell, 2004). Indian plants are the chief source of medicines and plant products. From eras until date, these medicinal plants have been widely utilized in Ayurveda.

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Recently, many such plants have been gaining importance due to their unique constituents and their useful applicability in various developing fields of research and development**.** Greensynthesis of nanoparticles also provideinnovation over other methods as they are modest, one step, cost effective, environment friendly and relatively reproducible and often results in more stable materials (Mittal, 2014). The use of plant extract for synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures (Njagi, 2011 and Zargar, 2011). *S. Viminalis*plant belongs to the family *Salicaceae* and genus *Salix*. It is *a* well-known hyper accumulator of cadmium, chromium, lead, mercury, Ag, uranium, and zinc (McCutcheon, 2003 and Schmidt, 2003). The syntheses of bioactive Ag nanoparticles from lichens were found to have higher antibacterial activity and antioxidant capacity and may be used as potential scavenger (Dasari, 2013). *S. Viminalis*is native to *Asia*, *Europe,* and *Himalayas* (Rushforth, 1999; Perttu and Kowalik, 1997). It is generally used for the treatment of arthritis, malaria, intestinal diseases and gout etc. This plant contained glycosides, flavonoids, phenols, and terpenoids (Alam, 2006). $AgNO_3$ is an inorganic compound and it is well known for possessing an inhibitory effect toward many pathogens and microorganisms commonly present in medical and industrial processes (Jiang *et al.,* 2004; Singhal *et al.,* 2011). Ag nanoparticles can be synthesized by numerous physical, chemical and biological methods. In medicines, Ag and Ag nanoparticles have an extensive application including skin ointments and creams containing Ag to avoid infection of burns and open wounds (Duran, 2005). The medicinal use of Ag is an antibiotic coating on medical devices. Ag and Ag nanoparticles are used as an antimicrobial in industrial, health care and domestic applications (Maillard, 2013). Ag nanoparticles are known for their outstanding antifungal, antibacterial, anti-viral and anti-inflammatory properties (Mulvaney, 1996). This work contributes experimental proof of the role of Ag^{+} and CTAB in the synthesis of Ag-nanoparticles from the dried leaves of *S. viminalis* plant at the room temperature. In our work we also focused on the evolution of antibacterial properties of the green synthesized Ag-nanoparticles. These green synthesized Ag-nanoparticles characterize with UV-vis, FT-IR, TEM, DLS.

MATERIALS AND METHODS

Materials

S. viminalis plant leaves were collected from Jammu, India. $AgNO_3$ supplied from Merck, India. CTABsupplied from Merck, India. Different bacterial species *E. coli,and S. aureus*were collected from H. Family Hospital, New Delhi, India.

Preparation of Plant Leaves Extract

Fresh leaves of *S. viminalis*were collected and rinsed carefully with tap water followed by distilled water to eliminate dust and harmful materials and then dried at room temperature. The aqueous extract were prepared by boiling 20 grams of cursed plant leaves in an Erlenmeyer flask with 200ml of distilled water for 5hrs at 30^0C followed by filtration.

Green Synthesis of Ag Nanoparticles

100ml 0.01mole/dm^3 solution of AgNO3 was prepared in an Erlenmeyer flask than 2, 3, 4 and 8 ml of plant extract were added individually in to AgNO3 solution keeping its concentration at 0.01mole/dm^3. Then 1ml of CTAB (cetyl tri methyl ammonium bromide) was also added in these solutions keeping its concentration at 0.01mole/dm^3. CTAB used as stabilizer in this work. We observed the reduction of Ag ion by measuring the absorbance of the reaction mixture within the range of 200nm to 800nm using UV-vis spectrophotometer. Synthesis of nanoparticles also established with solution color change from transparent to brown.

Characterization of ag nanoparticles

To detect the optical property of green synthesized Ag nanoparticles, samples were analyze for UV–vis spectroscopic studies (Hitachi U 3900) at room temperature, between 200 and 800 nm in range. Transmission electron microscopy was performed for characterizing the size and shape of green synthesized Ag nanoparticles. The samples were sonicated for 10 min. A drop of this solution was placed on carbon-coated copper grids. Size distribution of bio-reduced Ag nanoparticles was observed using Dynamic Light Scattering (DLS spectroscatter 201). FT-IR spectra were recorded on Perkin Elmer 1750 FTIR spectrophotometer.

Antibacterial Assays

The assessment of antibacterial activity was done using two different strains (*E. coli*&*S. aureus*). These microorganisms *E. coli* and *S. aureus* were used via standard disc diffusion method. Luria broth were used to cultivate bacteria and then incubated at 35^0C for 24hrs. 100µl of fresh overnight cultures were carefully placed on Luria agar plates to grow bacteria. Then 5mm sterile paper discs of green synthesized Ag nanoparticles placed in each plate for 24hrs and then measure the zone of inhibition for antibacterial activity.

Fig.1. Green synthesis of Ag-nanoparticles– visible observation due to color change from transparent tobrown

RESULT AND DISCUSSION

UV–vis absorbance studies

The addition of *S. viminalis* leaf extract to AgNO3 solution resulted in color change from transparent to pale yellow due to the fabrication of Ag nanoparticles as shown in figure1. These color changes arise because of the excitation of surface Plasmon vibrations with the Ag nanoparticles (Huang, 2010). We rely on that, formation of the absorption peak with a decrease in band width and increased in band intensity is a sign of spherical shaped, smaller particle with some agglomeration (Iravani, 2011). The slow but sure increase in temperature is a key feature suggesting an increase in the concentration of the Ag nanoparticles. Also, the frequency and the width of the surface Plasmon absorption the dielectric function of Ag practically disappeared (Korbekandi, 2012). Green synthesized Ag nanoparticles produced a peak within the range of 400 to 450 nm as shown in figure 2. UV–vis absorbance of reaction mixture was taken from 0 until 10 min. It was observed that absorbance peak indicating reduction of $AgNO_3$ into Ag- nanoparticles. It also observed that bio-reduction of Ag ions into nanoparticles started at the start of reaction and bio-reduction were completed at almost 10 min indicating rapid green synthesis of Ag- nanoparticles.

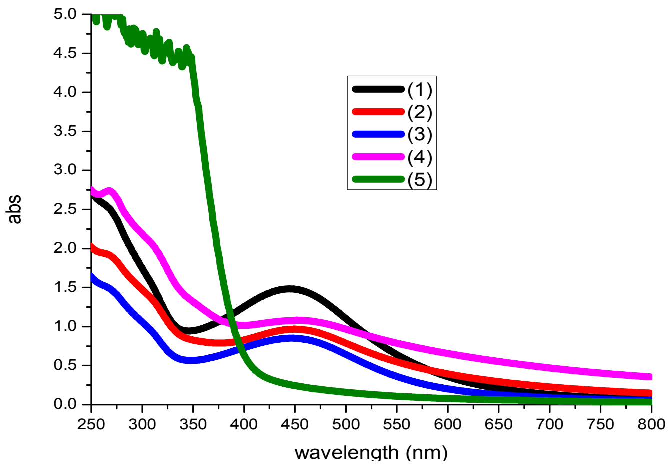

Fig. 2. UV-vis spectra of Ag nanoparticles.Reaction condition with constant $AgNO_3$ (10ml) and constant CTAB (1ml) with different amount of plant extract: [1] =2ml plant extract, [2] =3ml plant extract, [3] =4ml plant extract, [4] =8ml plant extract, [5]=plant extract

UV–VIS absorbance studies after three months

After approximately three months we again characterize our synthesized Ag nanoparticles with the help of UV-vis spectroscopy between the range of 200-700nm as shown in figure 3. Again synthesized Ag nanoparticles produced a peak within the range of 400 to 450 nm. This clearly indicate that our synthesized Ag nanoparticles quite stable for around three months.

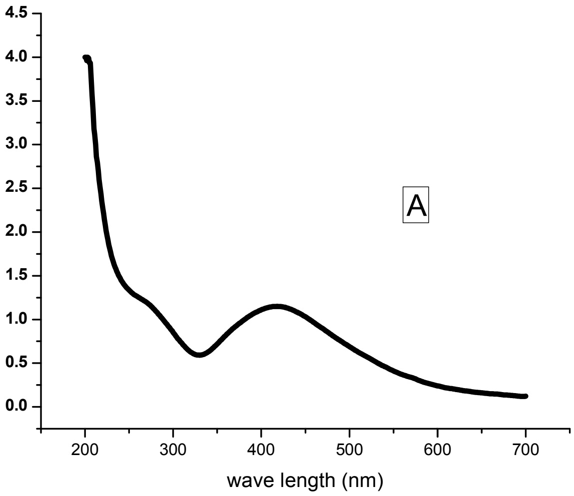

Fig. 3. UV-vis spectra of green synthesized Ag nanoparticles after nearly three months (A)

Transmission electron microscope (tem) studies

The TEM images of the prepared Ag nanoparticles are shown in figure 4. It observed that Ag nanoparticles were circular in shape with maximum particles in size range within 1-100nm. It also observed that Ag nanoparticles were equally distributed in the sample along with even shape.

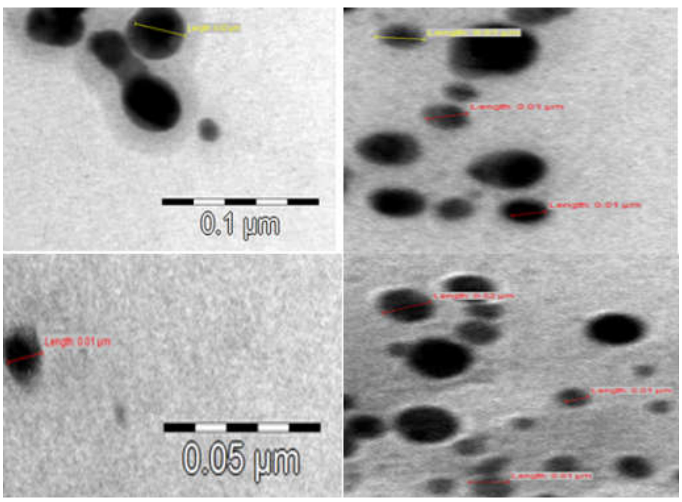

Fig. 4.TEM images of green synthesized Ag-nanoparticles at different magnification

Dynamic light scattering (dls) studies

The DLS graph tells that Ag nanoparticles synthesized by this method have a Zeta average diameter within the range of 1 to 100 nm as shown in Figure 5. The DLS calculated size is slightly different as compared to the particle size calculated from TEM because dynamic light scattering (DLS) method measures the hydrodynamic radius (Devi, 2015).

Fourier transform infra-red spectroscopy (ftir) studies

FTIR analysis was used for the characterization of the extract and the synthesized nanoparticles. In this work one peak showed at 1634 cm^{-1} corresponds to amide I arising due to -CO stretch in proteins present in plant extract as shown in figure 6. A band at 3302 cm^{-1} showed N-H stretching vibration of group -NH_2 and -OH the overlapping of the stretching vibration of due to water and *S. viminalis* leaves extract. Prepared extract sample showed a wide and strong peak with maximum intensity at 577 cm^{-1}. These IR spectroscopic studies revels that -CO group of amino acid residue have strong binding skill with metal signifying the formation of layer covering metal nanoparticles and also providing stability to the medium (Sathyavathi, 2010). These functional groups may have an effective role in the green synthesis of Ag nanoparticles. It may also say that flavonoids and terpenoids were responsible for reduction and effective stabilization of Ag nanoparticles.

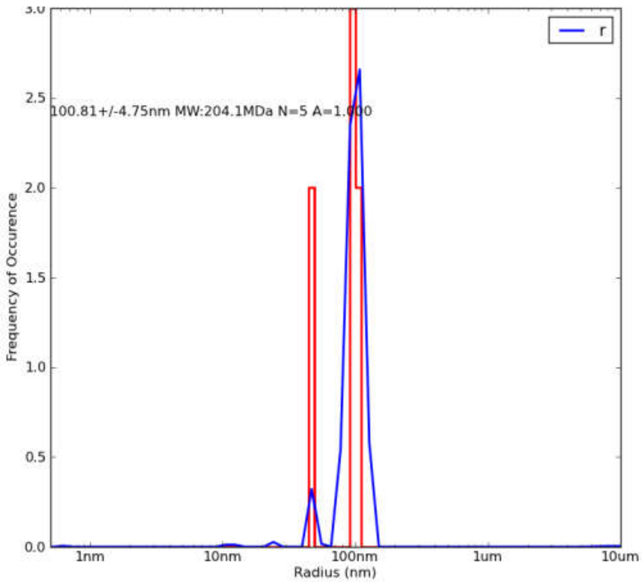

Fig. 5. Particle size distribution curve for green synthesized Ag-nanopaticles

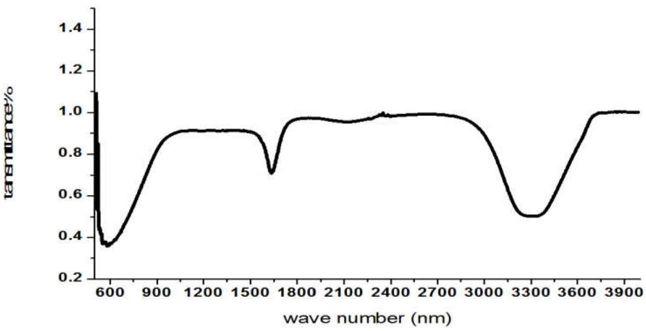

Fig. 6. FTIR spectra of green synthesized Ag-nanoparticles

Antibacterial Study

Since olden times, elemental Ag and its compounds have been used as antibacterial agents and were used to reserve water in form of Ag coins/Ag vessels (Padalia, 2014; Ahmed, 2015 and Kalishwaralal, 2010). Green synthesized Ag nanoparticles showed good anti-bacterial activity against the growth of pathogen. *E. coli* and *S. aureus* were found to be effective. It is clear from the homework, Ag nanoparticles by green synthesis can complete viable antibacterial agent used for the treatment of the bacterial infections. In this work, 0.01mole/dm^3 of the Ag nanoparticles was taken as final product for antibacterial assay. According to this wok the zone of inhibition increased with the increment of volume of Ag nanoparticles as shown in figure 7. The zone of inhibition increased with increasing the concentrations of Ag nanoparticles as 25%, 50% and 75% respectively. In the case of *E. coli* the maximum zone of inhibition (11mm) were obtained from 75% Ag-nanoparticles and 9mm zone of inhibition can be seen in 0.01mole/dm^3 AgNO3 solution. On the other side in the case of *S. aureus* the maximum zone of inhibition (7mm) were obtained from 75% Ag-nanoparticles and 5mm zone of inhibition can be seen in 0.01mole/dm^3 AgNO3 solution. But in the case of plant extract, no zone of inhibition was observed in both *E. coli* and *S. aureus*. So it can be determined that when we increase concentration of Ag nanoparticles, the zone of inhibition is also increased. The zone of inhibition of replicates of diameter were measured and to be tabulated in Table.1.

Table 1. Zone of inhibition of green synthesized Ag nanoparticles of *S. viminalis* Against *E. coli* and *S. aureus*

S. No.	Area of zone of inhibition in mm			
	E. coli		*S. aureus*	
1.	A	6mm	A	4mm
2.	B	6mm	B	5mm
3.	C	11mm	C	7mm
4.	D	9mm	D	5mm
5.	P.E	no zone	P.E	no zone

Fig.7. Antibacterial activity against *E.coli* (left) and *S.aureus* (right). (P.E) plant extract, (A) 25% Ag nanoparticles, (B) 50% Ag nanoparticles, (C) 75% Ag nanoparticles, (D) AgNO3 solution

Conclusion

This study shows that leaf extract of *S. viminalis* can be used efficiently for the bio-reduction of AgNO3 into Ag nanoparticles of average diameter within 1 to 100nm. The polyol components and the water-soluble heterocyclic components are responsible for the reduction of Ag ions and the stabilization of the nanoparticles**.** These synthesized Ag nanoparticles showed good antibacterial properties against both *E. coli* (gram -ve) and *S. aureus*(gram +ve). Synthesized Ag nano size distribution was determined by TEM and DLS and the optimum wavelength was recorded within the range of 400 to 450nm. So it also can conclude that this method is a rapid green synthesis, cost effective and having good antibacterial behavior. For future perspective this green chemistry approach toward the synthesis of Ag nanoparticles has many advantages such as, comfort with which the process can be mounted up, financial feasibility, etc. An application of such ecofriendly nanoparticles in bactericidal, wound healing and other medical applications makes this method possibly exciting for the large-scale synthesis of other Nano-materials. Synthesized Ag nanoparticles in this method are quite stable and remain intact for nearly three months if it protected under light proof conditions in dark room in the absence of sun light.

Declarations

Availability of data and materials: This section is not applicable in this manuscript.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: F K is my supervisor so she has great contribution in my manuscript like proof reading, grammar mistakes etc.

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